A METHOD FOR THE QUANTITATIVE DETERMINATION OF TRANSPIRATION IN PLANTS

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(WITH ONE FIGURE)

In plant-breeding work, which has engaged my attention for several years, a pressing need has been felt for some means of measuring the drought-resisting quality of individual plants. Morphological characters, such as small leaves, small and few stomata, pubescence, and thick epidermis, are characters which may be assumed to be correlated with drought resistance in that they tend to reduce transpiration and are characteristic of xerophytic plants in general. Moreover, it may be assumed that, other characters of two plants being equal, the one having the lower rate of transpiration per unit of leaf surface would be more suited to the drier portions of the plains region or to withstanding long periods of drought in the more humid districts. Some direct method of measuring the transpiration of plants growing in the field would be of great value therefore to the breeder in selecting for the quality of drought resistance.

At the beginning of our work in alfalfa breeding at the Kansas Experiment Station, no method was known to me which seemed to meet all of the requirements for securing data concerning transpiration for plant-breeding work. It was desired to use the plants selected as mother plants. Therefore they could not be taken up and planted, and the rate of loss of moisture determined by weight. Furthermore, on account of the very long tap roots of alfalfa, it would be impracticable to use a pot large enough to accommodate them without such pruning as would endanger the life of the plant, or at least make them little other than cut stems.

Although it is known that the cut stems of the plant when placed in water do not transpire normally, an attempt was first made to see if the differences sought could be revealed by this method. As a preliminary study and in order to ascertain the effectiveness of the potometer method in determining these individual differences in the

transpiration rate as compared with the same plants growing in the soil, four species were selected which seemed to promise a large range of difference in transpiration. Accordingly, two individuals of each of the following species were selected from young potted plants in the greenhouse: *Coleus Blumei*, *Chrysanthemum leucanthemum*, *Pelargonium* sp., and *Portulaca oleracea*. The plants to be used on their own roots were repotted into glass tumblers of suitable size. This was done without disturbing the root system. Evaporation from the soil was prevented by covering the tops of the glasses with a good quality of dental rubber.

For the potometers the plants were cut from the pots at the surface of the soil, one or two of the lower leaves removed, and the stems inserted, through holes in the cork, into bottles containing tap water. The whole stopper was then carefully sealed with paraffin. Each potometer was placed by the side of its corresponding potted plant and the whole series left on a table in a well-lighted room.

During the investigation, which lasted approximately sixty hours, the transpiration rate was found by weighings made at intervals of about one hour during the day. In Table I the average transpiration rate for each plant during the whole of the experiment is shown, and the transpiration rate of the plants on their own roots (normal) is compared with that of the plants in the potometer by reducing the latter to percentages of the former.

TABLE I

	Potted plants on own roots; transpiration in mg. per sq. cm. of leaf surface per hr.	Cut stems in potometer; transpiration in mg. per sq. cm. of leaf surface per hr.	Cut stems in potometer; per cent. of normal transpiration
Daisy	7.21	1.44	20.
Coleus	2.77	0.37	13.3
Portulaca	1.72	0.47	27.3
Geranium	0.65	0.65	100.

It will thus be noted that there is a great difference between the average transpiration rate of a plant on its own roots and that of a cut stem of the same plant placed in water. Moreover, roughly speaking, this difference is greatest in those plants having the highest normal transpiration. This difference, however, may vanish alto-

gether in plants with a low normal rate, as in the case of the geranium. A close scrutiny of Table I is sufficient to demonstrate that although the potometer will give some idea of the purely relative rates in different plants, it cannot be depended upon to give results which are at all comparable with their normal absolute transpiration rates. Thus if we arranged the plants according to their normal transpiration rates, they would stand from highest to lowest thus: daisy, coleus, portulaca, geranium; but if a similar arrangement were made from the results of the potometer experiment they would fall into the following order: daisy, geranium, portulaca, coleus. It was necessary therefore to find some other method for measuring transpiration whereby the plant could be kept on its own roots in the soil and in as nearly normal condition as possible.

As at least a partial solution of this problem, I would suggest the following method for measuring directly the transpiration of plants on their own roots, a method which provides at the same time for supplying them with a constant and uniform current of air. The method is based upon the well-known affinity of phosphorus pentoxid for water, whereby the two are combined and phosphoric acid formed. This compound has long been used as a drying agent and as a means of separating from air or other gases their water-vapor content, for the purpose of measurement. To this end known quantities of air are drawn through U-tubes containing P_2O_5.

Fig. 1 will show the method of setting up the apparatus. The apparatus consists essentially of a glass cylinder of suitable size to be used as a transpiration chamber, two U-tubes for P_2O_5, and an aspirator of known capacity. These are connected by rubber tubing, so that as the water flows from the aspirator a known quantity of air may be drawn through the cylinder and the pentoxid tubes. The cork in the top of the transpiration cylinder has two holes, one for the insertion of a thermometer and the other for the connecting tube to the pentoxid series. The cork for the bottom of the cylinder has two holes; through one is passed a short bent tube serving as an intake for the outside air; the other is to accommodate the stem of the plant. The cork is cut in halves, so that it may be fitted around the plant before inserting in the cylinder. If the stem be not large enough to fill the hole in the cork completely, this may be made close by packing

with vaselined absorbent cotton. The phosphorus pentoxid tubes used were 21^{cm} long and were fitted with glass stop-cocks and with suitable intake and outlet side tubes. The P_2O_5 was arranged in layers held apart by glass wool, so that the air could pass through it freely. Graduated aspirators fitted with stop-cocks at the base, and rubber stoppers at the top, would of course be more suitable,

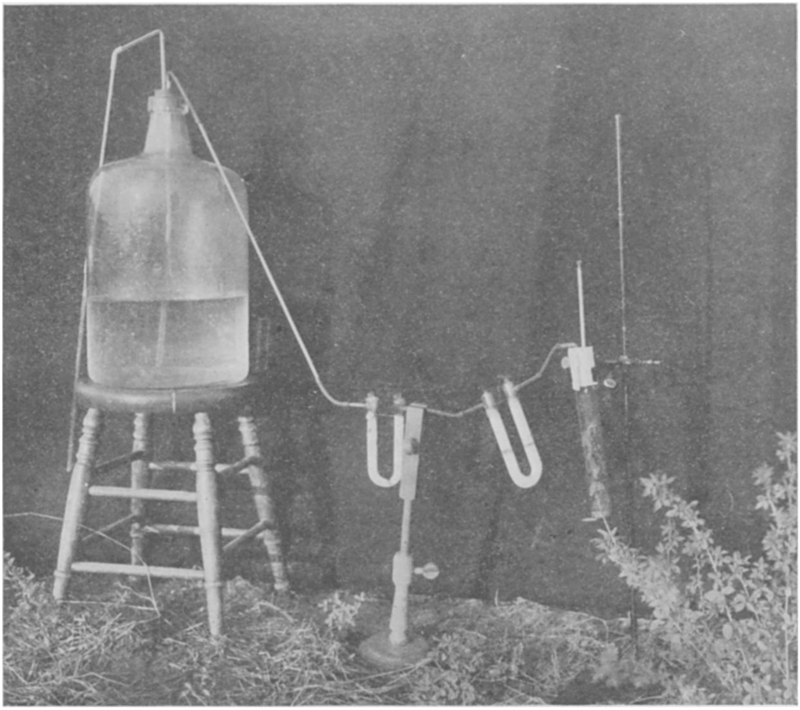

FIG. 1

but in the experiments I conducted 19.5 liter bottles, fitted with two-holed rubber stoppers, for the intake tube and the outflow water syphon, were used with satisfactory results.

For use the U-tubes are filled with P_2O_5, numbered and carefully weighed; the aspirator is filled with water and the whole apparatus set up, care being taken that all the connections are properly made and the joints air tight. The stop-cocks in the U-tubes are turned off, so that no moisture may reach the P_2O_5 between the time of weighing and

the beginning of the experiment. The last operation is the insertion of the stem of the plant into the cylinder. This should be done as quickly as possible and the exact time noted. The stop-cocks in the U-tubes are then turned so as to allow free passage of air, and the water is started running from the aspirator. As the water flows from the aspirator, a steady current of air is thereby drawn into the transpiration cylinder, where it passes over the leaves, out through the outflow tube at the top, thence through the pentoxid tubes, where both the moisture of the normal air and that given off by the plant is absorbed. One tube is usually quite sufficient if it is fresh, but since I used the same tube a number of times without refilling, it was thought best to use a second tube as a guard, and as a means of indicating the exhaustion of the water-absorbing capacity of the first tube. The flow of water from the aspirator was so regulated that each experiment lasted approximately an hour. The area of leaf surface, the cubic contents of the transpiration cylinder, and the capacity of the aspirator in the experiments made were approximately in the proportion of 1:5:500. Of course the leaf surface varied considerably in the different experiments, since it was impossible to obtain the same amount each time. It will thus be seen that the air in the transpiration cylinder was changed 100 times in 60 minutes, or once in every 36 seconds. The rise in humidity in the cylinder due to transpiration of the plant can therefore easily be controlled by regulating the rapidity of the flow of air through the cylinder or by changing the amount of leaf surface inclosed. The exact time when the water ceases to flow from the aspirator is noted, the stop-cocks in the U-tubes are cut off, and the plant severed from the parent stem at the point where it enters the cylinder. The plant may be kept in the transpiration cylinder until ready to be weighed. When the flow of air through the cylinder ceases, the contained air soon reaches the saturation point and the plant is thus kept fresh until ready for weighing and for putting the leaves in the press for area determination.

The increase in weight in the pentoxid tubes gives the sum of the amounts of water transpired by the plant in the given time, plus the water present in the quantity of air used; therefore it becomes necessary to know the exact water content of the air. This may be found by use of the sling psychrometer, the wet and dry bulb

thermometer, or by passing a given quantity of the normal air at the time of the experiment over phosphorus pentoxid in U-tubes in the same manner as in the case of the transpiration experiment itself. I prefer the latter method, as it is more direct and accurate, corrections are not required for altitude, barometric pressure, etc., and it calls for little extra labor to set up the additional apparatus and to run it simultaneously with the other. If all of the aspirators be of the same size, it will simply be necessary to subtract the increase in weight of the tubes used for the normal air from the increase in weight of those used in the plant series to find the actual amount of water vapor transpired by the plant in the given time. By dividing this amount by the product of the area of the leaves and the duration of the experiment expressed in minutes, and then multiplying this quotient by 60, the transpiration rate per hour per unit area is obtained.

The degree of accuracy of this method may be measured by checking it against the transpiration of a cut stem in a potometer or of a plant in a sealed pot. In my experiments I used the cut stem of an alfalfa plant. The leaves were removed from a few inches of the lower part of the stem, which was then inserted in a bottle of water through an opening in the cork which was thoroughly sealed over and around the stem with paraffin. By making several weighings of this plant before and after its use in the transpiration apparatus, its rate of loss of water was determined for the time before, during, and after its inclosure in the cylinder. The net increase in weight in the P_2O_5 tubes, after the weight of water present in the amount of normal air used has been subtracted from their total increase, should of course equal the loss in weight of the potometer during its inclosure in the cylinder. This method of checking was repeated three times with highly favorable results, details of which are shown in Tables II and III.

It will be noted from Table II that the last weighing of the potometer was made at 3:10, or two minutes before it was placed in the cylinder, and that the first weighing thereafter was made at 4:18, or five minutes after the air ceased to pass over the P_2O_5 tubes. The amount of water transpired during these intervals must be subtracted from the total of 0.150 grams as shown in Table II before comparing it with the net gain of the P_2O_5 tubes. Again referring to Table II, it

TABLE II

POTOMETER WEIGHINGS

Time	Total weight: grams	Number of minutes since last weighing	Loss: grams	Loss per min.: grams	Remarks
2:46 P. M.	108.890	....	......	......	
3:10 P. M.	108.775	24	0.115	0.0048	The rate in normal air before the experiment
4:18 P. M.	108.625	68	0.150	0.0022	Includes the period during which the plant was inclosed
4:48 P. M.	108.540	30	0.085	0.0028	The rate in normal air after the experiment

TABLE III

Plant placed in cylinder 3:12 P. M.	
Water stopped flowing from aspirator 4:13 P. M.	
Duration of experiment 61 minutes.	
Total gain in weight of P_2O_5 tubes	0.343 grams.
Amount of water in air used	0.217 grams.
Net gain of P_2O_5 tubes	0.126 grams.

will be seen that for the first of these intervals the rate was 0.0048^{gm} for two minutes or 0.0096^{gm}; and for the second, 0.0028^{gm} for five minutes or 0.0140^{gm}, making a total of 0.0236^{gm}. Now, 0.1500 $-$0.0236 $=$0.1264; it is thus seen that the two methods check within 0.004^{gm} for a one hour's run.

It would be useless to give the details of the other two check experiments, since they were carried on in exactly the same manner and extended through approximately the same length of time. It will suffice simply to show the results of the three experiments, corrected for time, as follows:

TABLE IV

	Transpiration as recorded by potometer	Transpiration as shown by P_2O_5 tubes	Difference
1st experiment	0.1264^{gm}	0.1260^{gm}	0.0004^{gm}
2nd experiment	0.0690	0.0660	0.0030
3rd experiment	0.0614	0.0624	0.0011

It will be noted again from Table II that the transpiration rate was greatly reduced during the time the plant was in the cylinder. This was of course due to the increase in relative humidity in the air, owing to the transpiration from the leaves. It may be suggested, therefore, that the plant was thus shown to be under abnormal conditions. However, the normal air in the room was at a temperature of 27° C. and contained 10.9^{mg} water per liter. According to the Smithsonian tables, such air at saturation contains 25.4^{mg} per liter; the relative humidity of the normal air was therefore 42.5 per cent. Since 19.5 liters of air were drawn through the transpiration cylinder, from which the P_2O_5 collected 343^{mg} of water, the air in the cylinder contained 17.5^{mg} per liter. This would give a relative humidity of 68.8 per cent. The change in relative humidity from the outside air to that inside the cylinder was thus seen to be from 42.5 per cent. to 68.8 per cent. This cannot be termed abnormal, since much greater changes in the outside air take place from day to day, and the range in a given day is very often even wider. Since the air was completely changed in the cylinder every thirty-six seconds, this condition of humidity must have been reached during that time, and have remained constant for the remainder of the hour. Owing to changes in temperature and light a plant in the open will vary much more in the transpiration rate in the course of a few hours than did the alfalfa plant when placed inside the cylinder. Until the exact effect of light, humidity, and temperature are known, therefore, and reduced to formulae whereby, the conditions of the given transpiration experiment being known, the transpiration constant for that plant can be estimated, all comparative transpiration experiments must be made at the same time, and under conditions as nearly identical as possible. The results of such experiments are moreover valuable only in proportion to the degree in which identical conditions are approached.

In order to test the practicability of this method in the field, I selected two alfalfa plants, growing within about 12^{dm} of each other, which showed marked differences in form and texture of the leaves. They had been previously cut at the same time and the stems were therefore of the same age and stage of growth, that is, just beginning to bloom. Three simultaneous comparative experiments were made on these two plants, in order to ascertain whether any constant

difference could be shown to exist in their transpiration rates per sq. cm. of leaf surface. The following table gives the details of this work. The column marked "check" contains the data derived from the apparatus set up to measure the quantity of water in the normal air.

An examination of Table V shows a constantly higher rate for no. 64 over that of no. 67; the rate of difference, however, was very variable. Unfortunately a larger leaf area was taken each time for no. 67, so much so that the relative humidity in its cylinder was higher than that of no. 64 in the first two experiments. This fact would place no. 67 at a disadvantage in these two experiments, since the higher relative humidity would retard to a greater degree its transpiration rate. Had it not been for the third experiment, therefore, in which, notwithstanding the greater leaf area of no. 67, the total transpiration of no. 64 was greater, thereby causing a higher relative humidity within its cylinder, the series would have been inconclusive. This third experiment, however, confirms the results of the other two, by showing that plant no. 64 may even overcome the disadvantage of a higher relative humidity and still transpire nearly twice as much per sq. cm. of leaf surface as the other.

It may be well to add here several suggestive details for those who wish to use this method. On account of the strong affinity of P_2O_5 for water, it must be kept very tightly sealed. Moreover, it is a difficult substance to handle, especially to transfer to the mouth of a small U-tube. I have found it very convenient to keep it in such a flask as is made especially for holding anhydrous copper oxid. This has a small neck out of which the P_2O_5 can readily be poured into the test tube, is fitted with a good ground-glass stopper within, and a ground-glass cap without. I have found that phosphorus pentoxid keeps perfectly dry in this form of bottle; the stopper does not stick fast, and it is ready for use at any time.

In filling the U-tubes it is well to push a bit of glass wool into the tube with a glass rod, pour on this about 0.5^{gm} of P_2O_5, then more wool and more P_2O_5, until the tube is filled. A bunch of glass wool is put on the top, so that in handling the tubes the P_2O_5 will not get into and clog the intake and outflow tubes while they are being handled, between the times of weighing and setting up the apparatus.

TABLE V

Plant no.	Date September	Experiment began	Experiment ended	Total gain in P_2O_5 tubes: mg.	Amount of air used: liters	Mg. of water in air used	Net gain of P_2O_5 tubes: mg.	Time: duration of experim't in minutes	Total number of leaves	Total area of leaves: sq. cm.	Loss mg. per hour per sq. cm. leaf surface	Relative humidity of air in cylinder	Relative humidity of external air	Temperature inside of cylinder	Temperature of external air
FIRST EXPERIMENT															
		P. M.	P. M.									%	%		
64.........	21	3:50	4:50	375	19.5	130	245	60	33	22.282	10.9	77.5	28.7	26°.5 C.	25° C.
67.........	21	3:51	4:55	418	19.5	130	288	64	14	32.457	8.3	86.4	28.7	26°.5 C.	25 C.
Check.....	21	3:48	4:51	130	19.5	....	....	63	....	......	....	....	....	.....	.....
SECOND EXPERIMENT															
		A. M.	A. M.												
64.........	23	10:16	11:26	324	19.5	148	175	70	12	17.130	8.7	59.8	28.	28°.5 C.	28 C.
67.........	23	10:22	11:29	353	19.5	148	205	67	14	41.981	4.3	65.3	28.	28°.5 C.	28 C.
Check.....	23	10:14	11:15	148	19.5	....	....	61	....	......	....	....	....	.....	....
THIRD EXPERIMENT															
		P. M.	P. M.												
64.........	23	4:13	5:29	422	19.5	126	295	77	29	21.252	10.9	80.4	25.3	28 C.	27 C.
67.........	23	4:16	5:40	415	19.5	126	289	84	11	33.810	6.1	79.1	25.3	28 C.	27 C.
Check.....	23	4:10	5:14	126	19.5	....	....	68	....	......	....	....	....	.....	.....

It is advisable to have a number of tubes already filled where work is being done in the field, in order that several parallel experiments may be run before returning to the laboratory for making weighings. The same tube may be used repeatedly so long as there is a part of it filled with dry P_2O_5. Care should be taken, however, to set up the tube in the same way each time, that is, to have the air enter and pass out of the tube in the same direction. The reason for this is that the phosphoric acid, after being formed from P_2O_5 plus water, will itself take up water. If now the tube be turned so that perfectly dry air in leaving the tube passes over the phosphoric acid, which has previously taken up an excess of water, it will itself take up water from the acid and the tube will lose weight.

It requires some experience to know how much leaf area to include in the cylinder for each experiment. After a few trials, however, one will learn to estimate sufficiently closely the capacity of the apparatus so as not to overcrowd it. Overfilling is indicated by the collection of moisture drops on the side of the cylinder, showing that the air inside has reached the saturation point. This may be remedied either by reducing the amount of leaf surface inclosed, or by increasing the rate of flow of the water through the aspirator.

This method of measuring transpiration may be said to be only a modification and a new adaptation of the methods used by LAMARLIÈRE[1] and E. and J. VERSCHAFFELT,[2] in that air is drawn over the plant in the same manner, and the transpired moisture collected in U-tubes containing hygroscopic substances which are not contained in the vessel with the plant, but are connected in the same aspirating series, so that the air, after passing through the evaporation cylinder, next passes through the U-tubes. However, a different absorbent is used and the apparatus, moreover, is adapted for measuring the transpiration of plants on their own roots. The essential point of difference, nevertheless, lies in the condition of the air as supplied to

[1] GANEAU DE LAMARLIÈRE, L., Recherches physiologiques sur les feuilles, developpées à l'ombre et au soleil. VI. Transpiration. Rev. Gén. Botanique **4**:529. 1892.

[2] VERSCHAFFELT, E. en J., De transpiratie der planten in koolzuurorije lucht. Botanisch Jaarboek (uitgegeven door het kruidkundig genootschap "Dodonaea" te Gent) **2**:305. 1890.

the plant. While in my method normal air is supplied, both of the above investigators first completely dried the air before allowing it to reach the plant. This is detrimental, for as BURGERSTEIN[3] says: "In Gegensatze zur zweiten hat die dritte Methode, bei welcher die evaporierte Wassermenge aus der Gewichtszunahme hygroskopische Substanzen in Erfahrung gebracht wird, den Nachteil das sich die Pflanze in einer zu trockenen Luft befindet."

SUMMARY

1. In plant breeding and in physiological and ecological work, it is very necessary to have some accurate and practical method of measuring the transpiration of plants.

2. The potometer method does not give the normal transpiration rate of a plant, neither can it be depended upon to give a rate which is even comparative as between different plants.

3. By the method herein described the transpiration of a plant under known and constant conditions may be accurately measured.

4. It is possible by this method to demonstrate individual differences in the transpiration rates in different plants of the same species.

5. By the use of this apparatus, data may be secured to serve as a basis for plant selection and breeding.

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3 BURGERSTEIN, ALFRED, Die Transpiration der Pflanzen 12. 1904.